I gotta see gene!
Seegene

DNA Walking *SpeedUp*™ Kit

SpeedUp **Sequencing**

SpeedUp **BAC Clone Sequencing**

SpeedUp **Genome Walking**

SpeedUp **Transgene Location Detection**

SpeedUp **Deletion/ Insertion/ Isoform Detection**

User Manual

Version 1.2
Published April 2004

Catalog No.:

DWSK-V101 (10 rxns), DWSK-V102 (25 rxns)

Storage Conditions: -20℃

For Research Use Only

Product Warranty and Liability

Seegene warrants the performance of all products as described when used according to instruction. Any problem incurred for any reason, other than misuse, should be reported to Seegene immediately. This warranty limits our liability to replacement of the products.

Safety Warning and Precautions

This product is limited for research use only, not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans nor animals.

Ordering Information and Technical Services

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The PCR process is covered by patents owned by Hoffman-La Roche Inc. No license or immunity under any other patent is either expressed or implied by the sale of any Seegene product.

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1. Introduction

As a third commercial application of Seegene's proprietary ACP (Annealing Control Primer) Technology maximizing PCR specificity, DNA Walking *SpeedUp*™ Kit is directed to the method using our unique DNA Walking ACP (DW-ACP) primer designed to capture unknown target sites and the optimized PCR conditions (referred as DW ACP-PCR™ technology hereunder). Due to the unique features of the DW-ACP primer system, DW ACP- PCR™ technology enables the researchers to obtain only genuine unknown target products up to the length (up to 2 kb) of which *Taq* polymerase is able to synthesize. This method provides the most powerful and revolutionary way to directly amplify unknown sequences adjacent to known sequences (**Figure1**). Whole genomic DNA, total RNA, cDNA or plasmid (clone) can be used as a starting material.

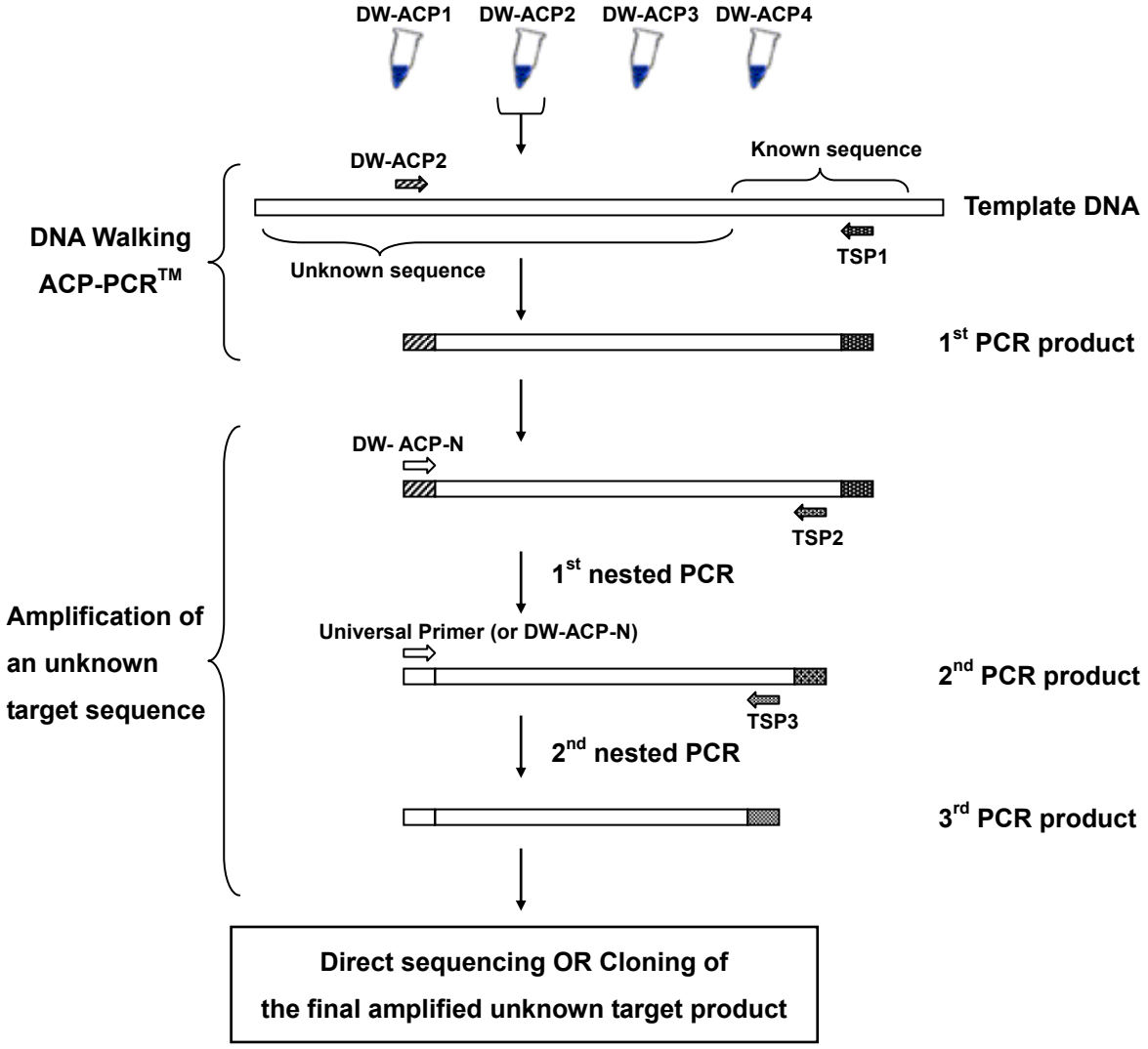


Figure 1. Flow chart of the general DNA Walking ACP-PCR™ Technology. DW-ACP and TSP denote **D**NA **W**alking-**A**nnealing **C**ontrol **P**rimer and **T**arget **S**pecific **P**rimer, respectively.

Applications:

(1) *SpeedUp* Sequencing

- Speed up sequencing of genomic DNA, cDNA, or plasmid
- Direct amplification and sequencing of unknown sequences flanking a known cloned sequence without subcloning or shotgun cloning
- Genomic sequence projects

DNA Walking *SpeedUp*™ Kit provides a simple and fast PCR-based method using our patented DW-ACP primer system for amplifying unknown sequences adjacent to known genomic DNA or cDNA sequences and providing templates for direct sequencing of the amplified unknown target DNA sequence.
Whole genomic DNA or single-strand cDNA generated by RT can be used as a template for direct amplification and sequencing or cloning of an unknown target sequence. The beauty of DNA Walking *SpeedUp*™ Kit comes from the ACP's maximized specificity along with the optimized two-stage PCR conditions.

(2) *SpeedUp* BAC Clone Sequencing

- Speed up sequencing of BAC clone
- Direct amplification and sequencing of BAC clone from BAC ends without subcloning or shotgun cloning
- Genomic sequence projects

DNA Walking *SpeedUp*™ Kit provides a simple and fast PCR-based method using our patented DW-ACP primer system for amplifying unknown sequences adjacent to known sequences (or BAC vector sequence) and providing templates for direct sequencing of the amplified unknown target DNA sequence.
Without shotgun cloning of BAC clone, BAC clone DNA can be used as a template for direct sequencing of the insert DNA. The beauty of DNA Walking *SpeedUp*™ Kit comes from the ACP's maximized specificity along with the optimized two-stage PCR conditions.

(3) *SpeedUp* Genome Walking

- Promoter region cloning or sequencing
- Gene structure (Exon/Intron junction)
- Gap filling
- Quick sequencing of larger size of DNA
- Transgene Location
- Deletion or insertion detection
- Splicing analysis

DNA Walking *SpeedUp*™ Kit provides a simple and fast PCR-based method using our patented DW-ACP primer system for amplifying unknown sequences adjacent to known genomic DNA sequences and providing templates for direct sequencing of the amplified unknown target DNA sequence.
This kit can be used for a variety of applications such as analysis of gene structure (exon/intron junction) or direct amplification and sequencing or cloning of unknown genomic DNA sequences. The beauty of DNA Walking *SpeedUp*™ Kit comes from the ACP's maximized specificity along with the optimized two-stage PCR conditions.

(4) *SpeedUp* Transgene Location Detection

- Speed up determination of location or orientation of a transgene in a transgenic organisms such as plant, animal, insect, fish, and bacteria
- Direct amplification and isolation of DNA fragments having a flanking region of a transgene using transgenic genomic DNA
- Direct amplification and cloning or sequencing of DNA fragments having a flanking region of a transgene

DNA Walking *SpeedUp*™ Kit provides a simple and fast PCR-based method using our patented DW-ACP primer system for determining the location or orientation of a transgene in transgenic organisms.
Since this kit allows the amplification of unknown sequences flankning a known transgene sequence, the insertion position or orientation of a transgene can be determined. Without cloning or library construction of transgenic genomic DNA, whole genomic DNA can be used as a template for direct screening of transgene location or orientation. The beauty of DNA Walking *SpeedUp*™ Kit comes from the ACP's maximized specificity along with the optimized two-stage PCR conditions.

(5) *SpeedUp* Deletion/ Insertion/ Isoform Detection

- Speed up detection of deletion, insertion, or isoform using genomic DNA or total RNA from experimental samples
- Direct isoform detection and isolation using total RNA without cloning or library screening or Northern blot hybridization
- Direct cloning of DNA fragments having known or unknown deletion or insertion mutation using genomic DNA
- Splicing analysis

DNA Walking *SpeedUp*™ Kit provides a simple and fast PCR-based method using our patented DW-ACP primer system for detecting deletion, insertion, or isoform by using whole genomic DNA or total RNA as a starting material.
This kit can be used to screen unknown mutations or unknown isoform as well as known mutations or known isoforms. Without cloning or library construction process, whole genomic DNA or first-strand cDNA can be used as a template for direct screening of deletion, insertion or isoform. The beauty of DNA Walking *SpeedUp*™ Kit comes from the ACP's maximized specificity along with the optimized two-stage PCR conditions.

Information of DNA Walking *SpeedUp*™ Kit

Product Name	Cat. #	Size
DNA Walking *SpeedUp*™ Kit	DWSK-V101	10 rxns
	DWSK-V102	25 rxns

2. List of Components

1 **2.5 μM DW-ACP1 primer for first PCR reaction**
DW-ACP1: 5'-ACP-AGGTC-3'

2 **2.5 μM DW-ACP2 primer for first PCR reaction**
DW-ACP2: 5'-ACP-TGGTC-3'

3 **2.5 μM DW-ACP3 primer for first PCR reaction**
DW-ACP3: 5'-ACP-GGGTC-3'

4 **2.5 μM DW-ACP4 primer for first PCR reaction**
DW-ACP4: 5'-ACP-CGGTC-3'

5 **10 μM DW-ACP-N primer for second PCR reaction**
DW-ACP-N: 5'-ACPN-GGTC-3'

6 **10 μM Universal Primer for third PCR reaction**
Uni-primer: 5'-TCACAGAAGTATGCCAAGCGA-3'

3. Storage Conditions

Store the reagents below -20℃.
Avoid multiple freeze/thaw.

4. Reagents and Equipment to be Supplied by User

Taq polymerase
2 mM dNTP
Target specific primers (TSP)
Micro-centrifuge
Thermal cycler
PCR purification kit

5. Protocol for DNA Walking *SpeedUp*™ Kit

<< Whole genomic DNA or cDNA >>

A. First PCR reaction (DNA Walking ACP-PCR™)

Firs PCR reaction in four individual tubes is performed independently using a primer pair each comprising the combination of DW-ACP1, 2, 3, or 4 and TSP1 primer.

1. Add the following reagents to a PCR tube **on ice**.

? μl	Whole genomic DNA or cDNA* (50-100 ng)
5 μl	10X buffer with 1.5mM $MgCl_2$
4 μl	2.5 μM DW-ACP (one of DW-ACP1, 2, 3, and 4)
1 μl	10 μM Target specific primer 1 (TSP1)
5 μl	2 mM dNTP
? μl	Distilled water
0.5 μl	*Taq* DNA polymerase* (5U/μl)
50 μl	Total volume

Note: We strongly recommend the use of ***Taq* DNA polymerase of Roche (Cat. No. 1418432) or Invitrogen (Cat. No. 10342) for the best results, but cannot guarantee the positive results with other *Taq* polymerases.**

2. Place the tube in a preheated (94℃) thermal cycler.
Note: It is important to preheat (94℃) the thermal cycler before placing the tube in.

3. Commence the PCR reaction immediately using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94℃	5 min
2	1	42℃	1 min
3	1	72℃	2 min
4	20 ~ 30	94℃	40 sec
		55℃	40 sec
		72℃	90 sec
5	1	72℃	7 min

Note: We recommend the GeneAmp PCR System 9700 of Applied Biosystems having a heated lid.

4. Purify the PCR products using PCR purification kit to remove the primers such as the DW-ACPs and the TSP1 used in the first PCR reaction.
   Note: We recommend the use of PCR purification kit (e.g., QIAGEN, Cat. No. 28106) for this step.
   Note: If you have a smearing problem in the third PCR reaction, you can dilute the first PCR products 10 fold or up to 100 fold.

B. Second PCR reaction (First nested PCR)

1. Add the following reagents to a PCR tube for the PCR reaction **on ice**.

1~2 μl	First PCR products
5 μl	10X buffer with 1.5mM $MgCl_2$
1 μl	10 μM DW-ACP-N
1 μl	10 μM Target specific primer 2 (TSP2)
5 μl	2 mM dNTP
? μl	Distilled water
0.5 μl	*Taq* DNA polymerase* (5U/μl)
50 μl	Total volume

   Note: We strongly recommend the use of ***Taq* DNA polymerase of Roche (Cat. No. 1418432) or Invitrogen (Cat. No. 10342) for the best results, but cannot guarantee the positive results with other *Taq* polymerases.**

2. Place the tube in a preheated (94℃) thermal cycler.
   Note: It is important to preheat (94℃) the thermal cycler before placing the tube in.

3. Commence the PCR reaction immediately using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94℃	3 min
2	30 ~ 35	94℃	40 sec
		60℃	40 sec
		72℃	90 sec
3	1	72℃	7 min

   Note: We recommend the GeneAmp PCR System 9700 of Applied Biosystems having a heated lid.

C. Third PCR reaction (Second nested PCR)

1. Add the following reagents to a PCR tube for the PCR reaction **on ice**.

1 μl	Second PCR products
5 μl	10X buffer with 1.5mM $MgCl_2$
1 μl	10 μM Universal primer
1 μl	10 μM Target specific primer 3 (TSP3)
5 μl	2 mM dNTP
? μl	Distilled water
0.5 μl	*Taq* DNA polymerase* (5U/μl)
50 μl	Total volume

Note: If you have a smearing problem in the third PCR reaction, the second PCR products can be diluted 10-100 fold by adding distilled water.

Note: We strongly recommend the use of ***Taq* DNA polymerase of Roche (Cat. No. 1418432) or Invitrogen (Cat. No. 10342) for the best results, but cannot guarantee the positive results with other *Taq* polymerases.**

Note: We recommend the use of DW-ACP-N if non-specific products are generated by using the Universal primer.

2. Place the tube in a preheated (94℃) thermal cycler.

Note: It is important to preheat (94℃) the thermal cycler before placing the tube in.

Commence the PCR reaction immediately using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94℃	3 min
2	35	94℃	40 sec
		60~65℃	40 sec
		72℃	90 sec
3	1	72℃	7 min

Note: We recommend the GeneAmp PCR System 9700 of Applied Biosystems having a heated lid.

3. Run 5-10 μl of the PCR products on 1.5-2% agarose gel stained with EtBr.

4. Extract the band on the agarose gel.
   Note: We recommend the use of GLASSMILK gel extraction kit (e.g., BIO 101, GENECLEAN II KIT) to extract your interest from the agarose.

5. Clone the extracted product into a TA cloning vector.
   Note: If you want to perform the sequencing directly without cloning step, you can commence the direct sequencing using universal primer or target specific primer (TSP).

<< Plasmid DNA >>

A. First PCR reaction (DNA Walking ACP-PCR™)

First PCR reaction in four individual tubes is performed independently using a primer pair each comprising the combination of DW-ACP1, 2, 3, or 4 and TSP1 primer.

1. Add the following reagents to a PCR tube **on ice**.

? μl	Plasmid DNA* (10-20 ng)
5 μl	10X buffer with 1.5mM $MgCl_2$
1 μl	2.5 μM DW-ACP (one of DW-ACP1, 2, 3, and 4)
1 μl	10 μM Target specific primer 1 (TSP1)
5 μl	2 mM dNTP
? μl	Distilled water
0.5 μl	*Taq* DNA polymerase* (5U/μl)
50 μl	Total volume

Note: We strongly recommend the use of ***Taq* DNA polymerase of Roche (Cat. No. 1418432) or Invitrogen (Cat. No. 10342) for the best results, but cannot guarantee the positive results with other *Taq* polymerases.**

2. Place the tube in a preheated (94℃) thermal cycler.
   Note: It is important to preheat (94℃) the thermal cycler before placing the tube in.

3. Commence the PCR reaction immediately using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94℃	5 min
2	1	42℃	1 min
3	1	72℃	2 min
4	20	94℃	40 sec
		55℃	40 sec
		72℃	90 sec
5	1	72℃	7 min

Note: We recommend the GeneAmp PCR System 9700 of Applied Biosystems having a heated lid.

4. Purify the PCR products using PCR purification kit to remove the primers such as the DW-ACPs and the TSP1 used in the first PCR reaction.

Note: We recommend the use of PCR purification kit (e.g., QIAGEN, Cat. No. 28106) for this step.

Note: If you have a smearing problem in the third PCR reaction, you can dilute the first PCR products 10 fold or up to 100 fold.

B. Second PCR reaction (First nested PCR)

1. Add the following reagents to a PCR tube for the PCR reaction **on ice**.

1~2 μl	First PCR products
5 μl	10X buffer with 1.5mM $MgCl_2$
1 μl	10 μM DW-ACP-N
1 μl	10 μM Target specific primer 2 (TSP2)
5 μl	2 mM dNTP
? μl	Distilled water
0.5 μl	*Taq* DNA polymerase* (5U/μl)
50 μl	Total volume

Note: We strongly recommend the use of ***Taq* DNA polymerase of Roche (Cat. No. 1418432) or Invitrogen (Cat. No. 10342) for the best results, but cannot guarantee the positive results with other *Taq* polymerases.**

2. Place the tube in a preheated (94℃) thermal cycler.

Note: It is important to preheat (94℃) the thermal cycler before placing the tube in.

3. Commence the PCR reaction immediately using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94℃	3 min
2	20	94℃	40 sec
		60℃	40 sec
		72℃	90 sec
3	1	72℃	7 min

Note: We recommend the GeneAmp PCR System 9700 of Applied Biosystems having a heated lid.

C. Third PCR reaction (Second nested PCR)

1. Add the following reagents to a PCR tube for the PCR reaction **on ice**.

1 μl	Second PCR products
5 μl	10X buffer with 1.5mM $MgCl_2$
1 μl	10 μM Universal primer
1 μl	10 μM Target specific primer 3 (TSP3)
5 μl	2 mM dNTP
? μl	Distilled water
0.5 μl	*Taq* DNA polymerase* (5U/μl)
50 μl	Total volume

Note: If you have a smearing problem in the third PCR reaction, the second PCR products can be diluted 10-100 fold by adding distilled water.

Note: We strongly recommend the use of ***Taq* DNA polymerase of Roche (Cat. No. 1418432) or Invitrogen (Cat. No. 10342) for the best results, but cannot guarantee the positive results with other *Taq* polymerases.**

Note: We recommend the use of DW-ACP-N if non-specific products are generated by using the Universal primer.

2. Place the tube in a preheated (94℃) thermal cycler.

Note: It is important to preheat (94℃) the thermal cycler before placing the tube in.

Commence the PCR reaction immediately using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94℃	3 min
2	35	94℃	40 sec
		60~65℃	40 sec
		72℃	90 sec
3	1	72℃	7 min

Note: We recommend the GeneAmp PCR System 9700 of Applied Biosystems having a heated lid.

3. Run 5-10 μl of the PCR products on 1.5-2% agarose gel stained with EtBr.

4. Extract the band on the agarose gel.
   Note: We recommend the use of GLASSMILK gel extraction kit (e.g., BIO 101, GENECLEAN II KIT) to extract your interest from the agarose.

5. Clone the extracted product into a TA cloning vector.
   Note: If you want to perform the sequencing directly without cloning step, you can commence the direct sequencing using universal primer or target specific primer (TSP).

6. Troubleshooting Guide

Problems	Comments
No band	a. You may have a problem with primer design. Re-design your target specific primers. b. Reduce the annealing temperature. c. Extend the length of extension. d. Check the quality of template DNA. e. Your target DNA may have a GC-rich region. f. Retry the PCR reaction using long-distance DNA polymerase.
Multiple bands	a. Your interest may be multicopies. b. You may have a problem with primer design. Re-design your target specific primers. c. The template DNA may be contaminated. d. If you use transgenic DNA, transgene may have multiple copies in different genomic DNA loci. e. It is critical to set up the PCR reaction on ice before samples are placed in the thermal cycler. f. Retry the PCR reaction using other thermostable DNA polymerase.
Smearing	a. Check the quality of template DNA or primer oligonucleotides. b. You may have a problem with the concentration of the first and second PCR products used in each nested PCR reaction. Have the first and second PCR products diluted up to 100 or more fold. c. You may have a problem with primer design. Re-design your target specific primers. d. Retry the PCR reaction using other thermostable DNA polymerase. e. It is critical to set up the PCR reaction on ice before samples are placed in the thermal cycler.

Appendix A. Primer Design

You have to design target specific primers (TSP) for DNA Walking ACP-PCR™ reactions. You may have to design two nested primers for obtaining real products.
The primers should be:

22 ~ 25 nucleotides long
GC content > 50%
TSP1 **55℃≤Tm≤60℃**
TSP2, TSP3… **60℃≤Tm≤65℃**

The primers should have a GC content of 50 ~ 70%. The primers should not be able to form secondary structures due to internal complementarities. Avoid containing sequences at the 3'-end that allow base pairing with itself or other primer. Avoid repetitive sequence or regions containing stretches of the same nucleotide.
Sometimes, the specificity of your PCR reaction may be low (high level of nonspecific background), resulting in mispriming and the generation of false amplification products. In this case, design nested primers to amplify an internal region of the original amplified product. Nested PCR increases specificity and sensitivity by reducing the nonspecific products.

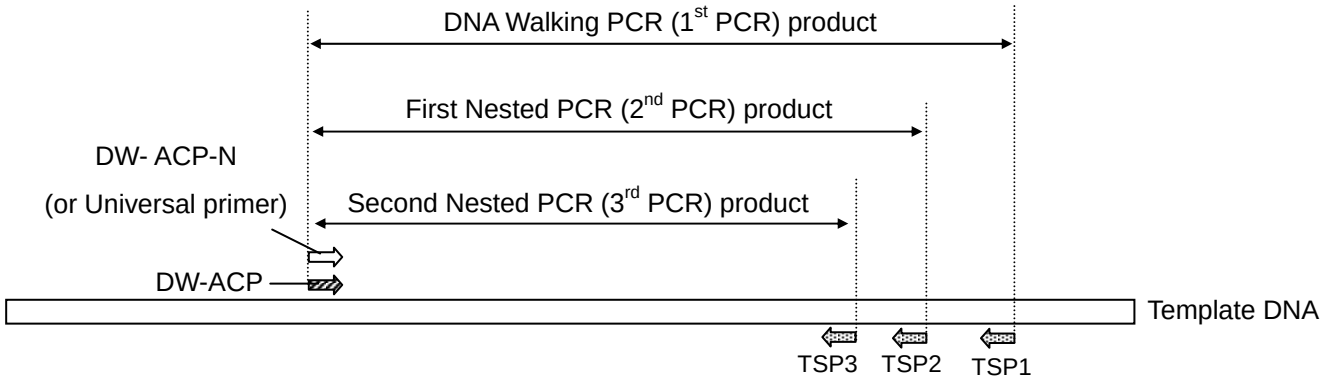


Figure 2. The diagram of DNA Walking using DNA Walking ACP-PCR™ Technology. The spotted arrows indicate target specific primers (TSPs). Nested primers are designed to amplify an internal region of the original amplified product. The TSP2 and TSP3 indicate nested primers.

Appendix B. Expected Results

The control experiments were conducted using mouse (ICR) cDNA, genomic DNA or bacteria genomic DNA. Three target specific primers (TSP1, 2 and 3) for each gene were designed to amplify unknown target sequences adjacent to the known sequences. First PCR reaction was performed using DW-ACPs and TSP1 primer as described in section 5A. In the second and third PCR reactions, DW-ACP-N and TSP2, and Universal primer and TSP3 were used as described in sections 5B and 5C, respectively.

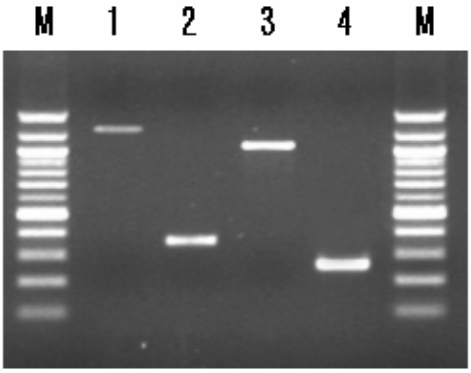


Figure 3. The amplification of the 5'-end region sequences of PBP cDNA using DNA Walking *SpeedUp*™ Kit. Each of four different DW-ACP1 (lane 1), DW-ACP2 (lane 2), DW-ACP3 (lane 3), and DW-ACP4 (lane 4) generated one major product showing a different size. These products were turned out to be the 5'-end region sequences of PBP cDNA by sequence analysis. These results indicate that the DNA Walking *SpeedUp*™ kit can be applied to amplify the unknown sequences adjacent to a known partial cDNA sequence.

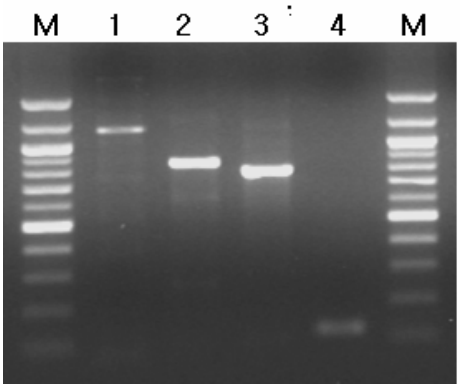


Figure 4. PCR amplification products for mouse TNF-α promoter region using DNA Walking *SpeedUp*™ Kit. Each of four different DW-ACP1 (lane 1), DW-ACP2 (lane 2), DW-ACP3 (lane 3), and DW-ACP4 (lane 4) generated one major product showing a different size. These results turned out to be the promoter sequence of the TNF-α gene by sequence analysis. M: Forever 100bp Ladder Personalizer

Expected Results, continued

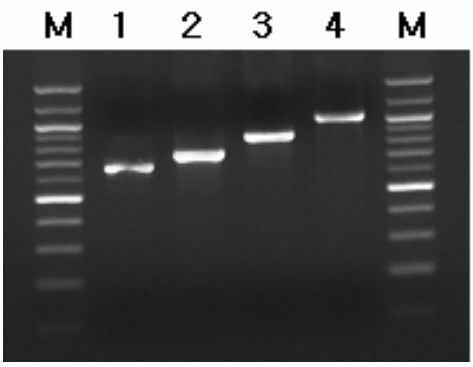
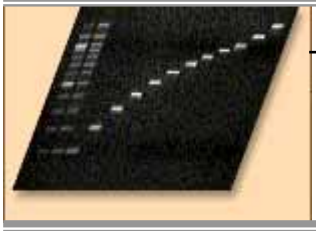


Figure 5. PCR amplification products for bacteria argC promoter region using DNA Walking *SpeedUp*™ Kit.
M: Forever 100bp Ladder Personalizer, Lanes 1~4: one major product generated by each different DW-ACP primer (DW-ACP1, 2, 3, and 4) from upstream of the argC gene from bacteria genomic DNA.

Related Products



Forever 100bp Ladder Personalizer

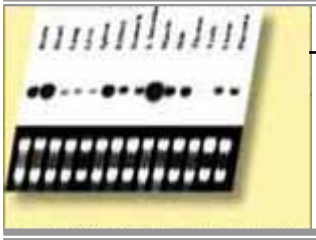
Our unique 100 bp endless usage ladder system (patent pending) is clearly distinguished from any existing commercialized consumables 100 bp DNA ladder. This system supplies templates (plasmids) which will be amplified to be used for size markers.

GeneFishing™ DEG kits

All of the GeneFishing™ DEG kits (DEG101~106) comprise 20 randomly selected arbitrary ACPs (Annealing Control Primers) and each DEG kit works equally for your target samples.

Full-length cDNAs

Seegene's Full-length cDNAs are ideal to study gene expression in specific tissues and at specific developmental stages and also to clone the genes belonging to a multigene family.

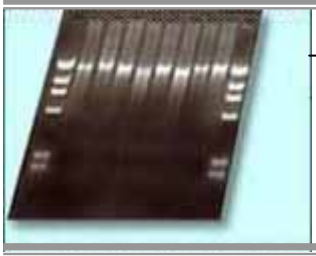


Pre-made Northern Blots

Northern blots are pre-made for immediate use and designed to See Gene expression in specific tissues and at specific developmental stages. Our Northern blots allow you to assess the distribution, size, alternative splicing forms, and level of your transcripts in one experiment.

Zoo Blot

We are offering zoo blot(pre-made Southern blot) including 12 different species for your screening assays. Genomic DNAs were prepared from human, rat(SD), mouse(ICR), dog, cow, pig, rabbit, chicken, frog(Xenops), fish(Zebra fish), C. elegans, and yeast.



Genomic DNAs

We are offering genomic DNAs obtained from 12 different sample sources for your screening assays. Seegene's genomic DNA is qualified for genomic analysis including PCR and library construction

Seegene's Distributors

Australia

Scientifix Pty Ltd
PO Box18 Southland Centre, Cheltenham Victoria
Tel: 61 3 9462 7488 Fax: 61 3 9548 7177
Free call: 1800 007 900
E-mail: info@scientifix.com.au
URL: www.scientifix.com.au

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Note:

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